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SCANNING ELECTRON MICROSCOPY FOR THE STUDY OF THE WINTER STONEFLY GENUS *CAPNIA* (PLECOPTERA: CAPNIIDAE)

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Abstract.—Scanning electron micrographs are produced for nine species of *Capnia*: *C. barberi, C. cheama, C. coloradensis, C. decepta, C. elevata, C. fibula, C. manitoba, C. melia,* and *C. uintahi.* Structural details not previously reported such as spines and sensilla may prove useful in constructing future phylogenies. The value of scanning electron microscopy for species identification and systematic work is discussed.

Scanning electron microscopy (SEM) has been used to examine structural details of Plecoptera eggs (Baumann, 1973; Stark and Baumann, 1978; Szczytko and Stewart, 1979; Stark and Stewart, 1981; Stark and Szczytko, 1981a, b; Stark and Stewart, 1982c; Stark and Ray, 1983; Stark, 1983; Stark and Szczytko, 1982; Stark and Szczytko, 1984), mouthparts (Baumann, 1973; Stark and Stewart, 1981; Stark and Stewart, 1982a–c; Stark and Ray, 1983), and sensilla (Stark and Stewart, 1981; Stark and Stewart, 1982a; Stark and Ray, 1983; Stark, 1983; and Kapoor and Zachariah, 1984). These studies have helped in constructing phylogenies such as those of Nelson (1984). The use of SEM to illustrate male genitalia of stoneflies has been limited to the species description of *Prostoia hallasi* (Kondratieff and Kirchner, 1984). A study of the male genitalia of *Capnia* was undertaken to examine the feasibility of using SEM as a tool to differentiate species and species groups in this large genus.

Materials and Methods

Specimens of North American *Capnia* were examined and compared to holotypes (except that of *C. fibula* Claassen, which was missing). The term genitalia refers to the epiproct of the male and associated sclerites while terminalia refers to all the appendages of the abdominal segments including tergal knobs, genitalia, and cerci. Collection data for the specimens used in this study are:

Capnia barberi Claassen—CALIFORNIA, Nevada County, Sagehen Creek, 26 February 1965, Sheldon and Hawthorne.

Capnia cheama Ricker—MONTANA, Lincoln County, Kootenai River, 19 March 1970, R. L. Newell.

Capnia coloradensis Claassen—COLORADO, Routt County, Fish Creek, Hwy 40, Steamboat Springs, 6 April 1963, A. R. Gaufin.

Capnia decepta Banks—ARIZONA, Cochise County, Huachuca Mountains, Ramsey Canyon, 17 January 1984, Baumann and Nelson.

Capnia elevata Frison—OREGON, Wasco County, creek, Hwy 30, 4 mi E Rowena, 3 March 1984, Baumann and Nelson.

Capnia fibula Claassen—ARIZONA, Coconino County, West Fork Oak Creek, 6 February 1984, M. W. Sanderson.

Capnia manitoba Claassen—MAINE, sta. 2 Rt. 5 Saco, 3 April 1964, C. H. Nelson.

Capnia melia Frison—OREGON, Clackamas County, Salmon River at headwaters, jct. Hwys 26 & 35, 2 March 1984, Baumann, Nelson, Fiala.

Capnia uintahi Gaufin—UTAH, Davis County, Farmington Canyon at Fork Bridge, 5 April 1966, R. W. Baumann.

In preparation for study with the scanning electron microscope, specimens were serially dehydrated from their storage fluid of 70% alcohol for more than 10 minutes in 95% ethanol, 20 minutes in absolute (100%) ethanol over calcium sulfate, and stored for more than 20 minutes in acetone previously kept over calcium sulfate. The specimens were then transferred to acetone-immersed holding baskets in a Sorvall critical point drying apparatus attached to a carbon dioxide tank. The dried abdomens of the specimens were then placed on aluminum stubs covered with double-sided tape. The specimen-bearing stubs were gold coated using a Polaron gold-coating apparatus. Coated specimens were then examined using an AMRay 1000 scanning electron microscope at 20 kV.

Results

This study reveals that male terminalia of *Capnia* are well suited for examination using SEM. The epiproct, which is used in species determinations, is heavily sclerotized and thus resistant to deformation during drying and other preparation procedures necessary for viewing with a scanning electron microscope. This structural stability in the *Capnia* is uncommon in stoneflies since important taxonomic characters in genera in other families are usually on softer body parts that are susceptible to deformation during specimen preparation.

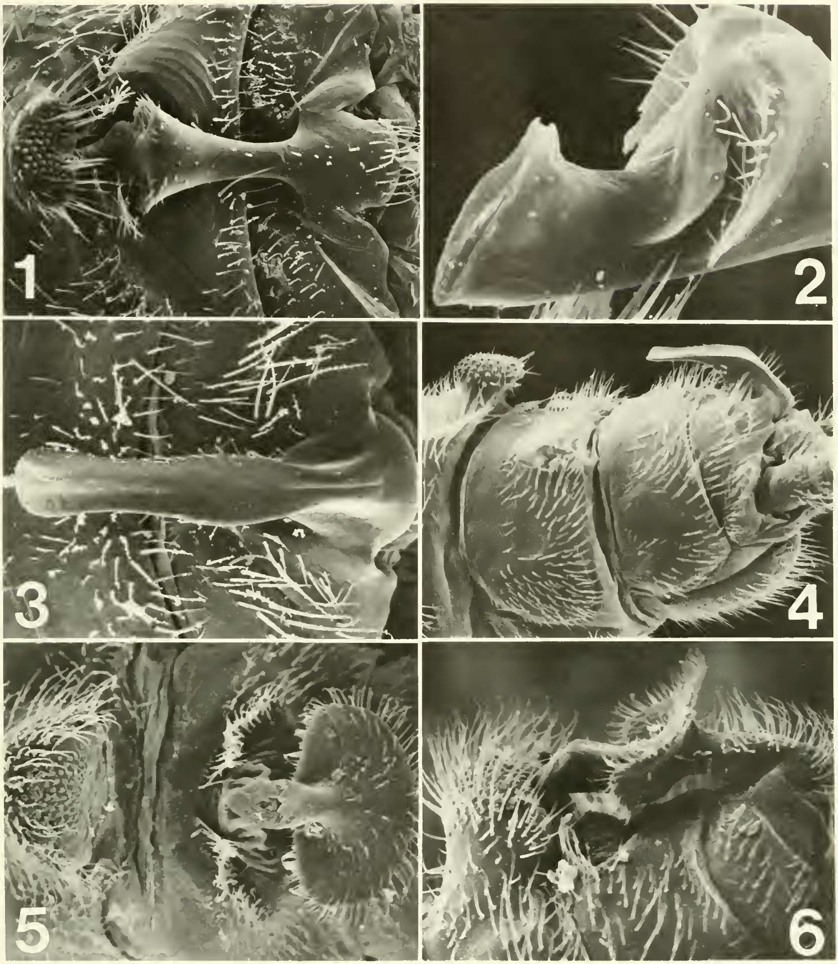
Detailed structures not previously noted in species descriptions were seen with the SEM. A few of these features that indicate phylogenetic relationships are outlined below.

In *C. cheama* (Fig. 1) and *C. fibula* (Figs. 4 and 12) details of the dorsal abdominal hump are shown. Both trichoid and campaniform sensilla are present. The paucity of trichoid sensilla on the knob of tergum seven in *C. fibula* compared with the numerous sensilla of the knob of tergum eight in *C. cheama* distinguishes two groups of species in the genus. In *C. excavata* and *C. uintahi* the setation of the tergal knob resembles that of *C. cheama* and the setation of *C. fibula* is similar to that of *C. venosa, C. elevata, C. wanica,* and *C. manitoba.*

In *C. fibula* (Fig. 3) spines are asymmetrical along the lateral margins of the epiproct when seen in dorsal view. These spines are directed cephalad and would seemingly hinder insertion of the epiproct into the female. Similar spines occur in several species groups of *Capnia* and take on a diversity of placements including the stout, tightly bunched configuration of *C. decepta* (Figs. 11, 13 and 15) and a scattered pattern along the anterior margin of the epiproctal bulb of *C. melia* (Figs. 14 and 16). Other species such as *C. barberi* (Fig. 9) show no indication of these spines.

The two-limbed epiproct of *C. manitoba* (Fig. 8) probably represents a plesiomorphic condition for at least one group of *Capnia.* The presence of a lower epiproctal limb was first noted using SEM in *C. elevata* (Fig. 10) and later noted using a light microscope in *C. fibula, C. venosa,* and *C. wanica.* These species show a reduced lower process and a cline featuring a gradual reduction in the length of this character as one moves away from the range of *C. manitoba.* No other named species of *Capnia* in North America has a similar two-limbed epiproct or hint of a reduced lower limb.

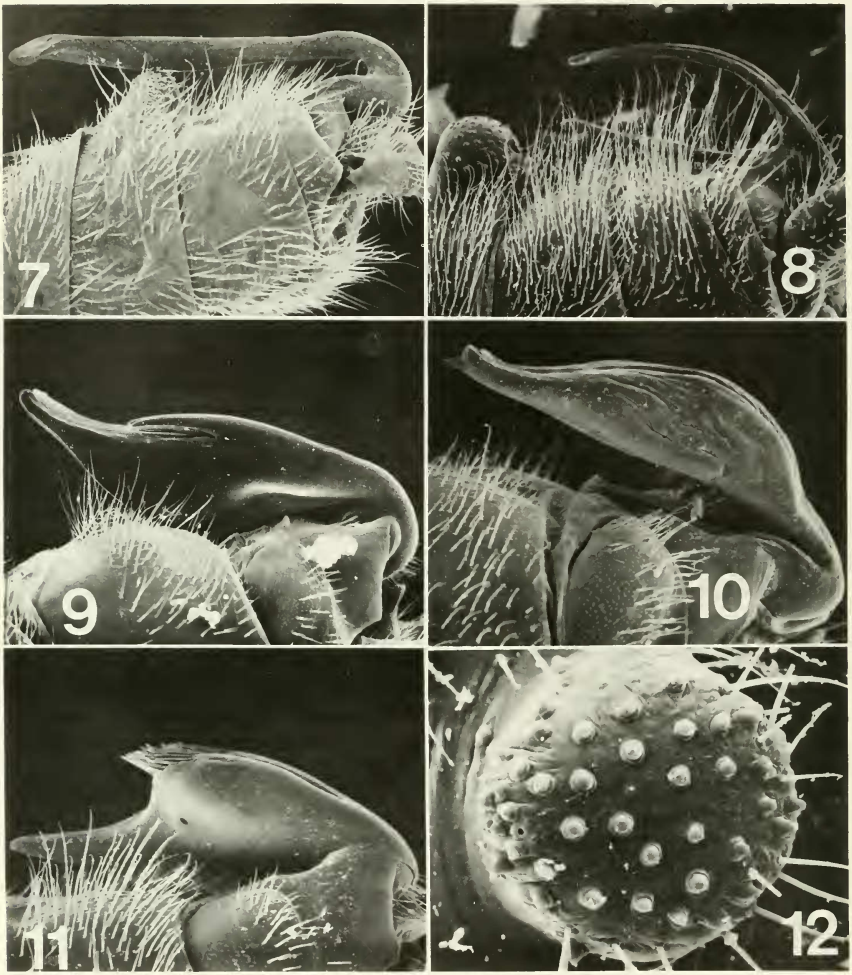
A relatively simple tube epiproct such as that of *C. coloradensis* (Fig. 7) is shared by several species in the genus, but only one other, *C. petila,* has the apex of the epiproct drooping ventrally. The tube epiproct of some *Capnia* represents the most apomorphic condition of the epiproct and is shared by several species, including *C. con-*



Figs. 1–6. Male terminalia of *Capnia* spp. 1, *C. cheama* Ricker, epiproct and tergal knob, 225× (dorsal). 2, *C. cheama* Ricker, tip of epiproct, 650× (anterolateral). 3, *C. fibula* Claassen, epiproct, 180× (dorsal). 4, *C. fibula* Claassen, epiproct and tergal knob, 75× (lateral). 5, *C. uintahi* Gaufin, epiproct and tergal knob, 105× (dorsal). 6, *C. unitahi* Gaufin, epiproct and tergal knob, 110× (lateral).

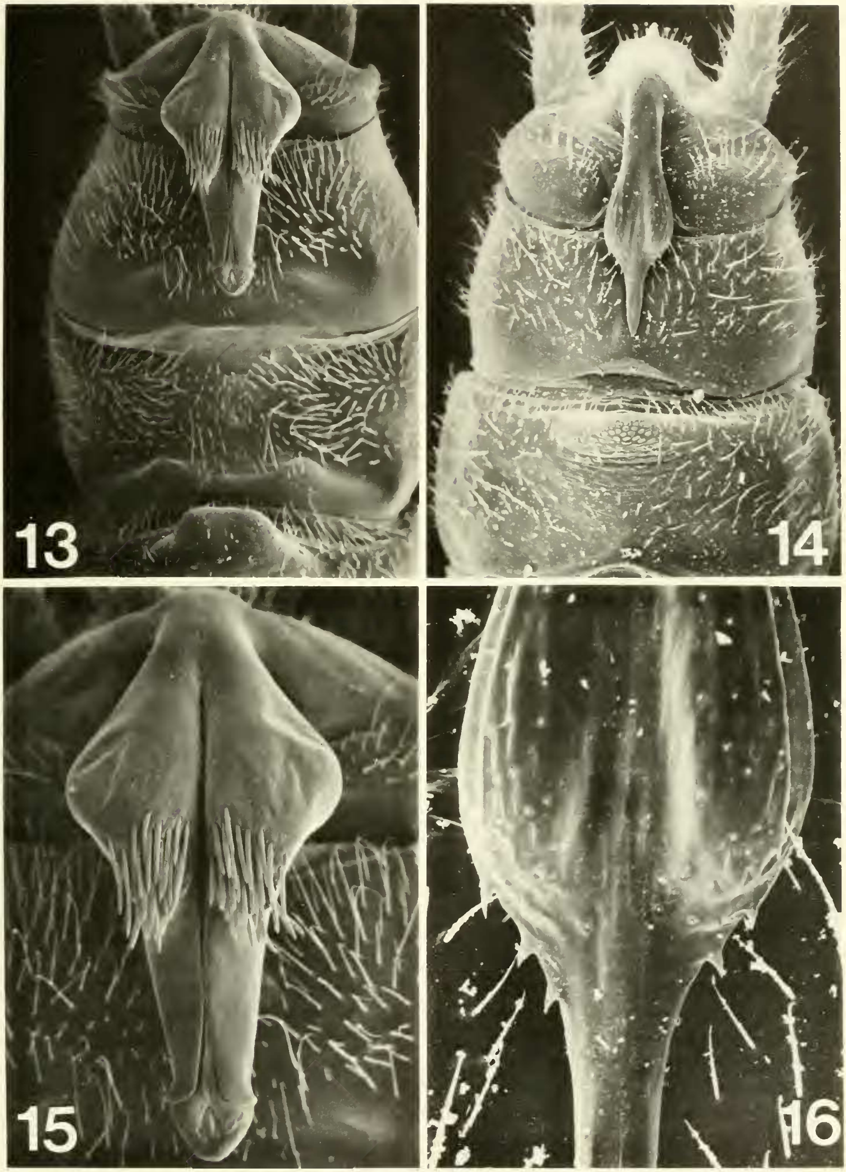
fusa, C. elongata, C. gracilaria, C. lacustra, C. lineata, C. promota, C. vernalis, and *C. zukeli.*

Two closely related species, *C. cheama* and *C. uintahi* demonstrate the value of SEM in illustrating characters useful for determinations at the specific level. The distribution of the two kinds of sensilla on the



Figs. 7–12. Male terminalia of *Capnia* spp. 7, *C. coloradensis* Claassen, epiproct and tergal knob, 105× (lateral). 8, *C. manitoba* Claassen, epiproct and tergal knob 120× (lateral). 9, *C. barberi* Claassen, epiproct, 90× (lateral). 10, *C. elevata* Frison, epiproct, 105× (lateral). 11, *C. decepta* Frison, epiproct, 100× (lateral). 12, *C. fibula* Claassen, tergal knob, 700× (dorsal).

tergal knobs (Figs. 1 and 5) are useful in separating these two species. Higher magnification of the tip of the epiproct (Figs. 2 and 6) shows a dorsal appendage near the apex in *C. cheama* which is not present in *C. uintahi.* These structures are not readily apparent using a dissecting microscope and phylogenetically important information given by these characters has been unavailable using classical techniques.



Figs. 13–16. Male terminalia of *Capnia* spp. 13, *C. decepta* Frison, epiproct and tergal knob, 80× (dorsal). 14, *C. melia* Frison, epiproct, 150× (dorsal). 15, *C. decepta* Frison, epiproct, 85× (dorsal). 16, *C. melia* Frison, epiproct, 800× (dorsal).

Discussion

The higher magnification and greater depth of field available make SEM a valuable tool for investigating evolutionary and taxonomic relationships within *Capnia* and the potential for greater use is apparent. Comparison of minute details between taxa can aid systematists in understanding species variation and constructing accurate phylogenies. The expense of SEM work and the often destructive manipulation of specimens, though, limit usefulness of SEM for routine species determination. These constraints could result in overconfidence based on the characters observed from a single specimen rather than from a series of specimens. However, once a new character is observed using SEM more specimens may be examined using a dissecting microscope to confirm the character from a series of individuals.

Although relatively low magnification was used during this preliminary study, other structures of probable taxonomic worth, such as epiproctal spines, sensilla, and setation patterns were observed. Once the minute characters are examined from series of specimens and relationships established, routine species determinations can be performed using characters visible with an ordinary dissecting microscope.

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